Centers for Birth Defects Research and Prevention

The National Birth Defects Prevention Study Protocol

Table of Contents

Centers for Birth Defects Research and Prevention

The National Birth Defects Prevention Study Protocol

1. GENERAL DESCRIPTION AND OVERVIEW

Birth defects are the leading cause of infant mortality in the United States, accounting for 21% of all infant deaths in 1995. In addition, birth defects are the fifth leading cause of years of potential life lost and contribute substantially to childhood morbidity and long-term disability. Although several human teratogens have been identified, most birth defect cases have an unknown etiology. Surveillance systems can be used extensively to identify birth defects risk factors, as well as to identify unusual patterns of birth defect occurrences. However, because individual birth defects are relatively rare, it has been difficult in the past to conduct a study large enough to provide the necessary power to evaluate the causes of specific defects.

The Centers for Birth Defects Research and Prevention (CBDRP) is a collaborative effort between the CDC's National Center on Birth Defects and Developmental Disabilities (the CDC's Metropolitan Atlanta Congenital Defects Program) and nine birth defects surveillance registries across the United States (Arkansas, California, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas and Utah). This collaborative effort provides a unique and unprecedented opportunity to evaluate risk factors for birth defects. Major strengths include: 1) large population-based birth defects registries including populations with diverse environments; 2) improved case definition (classifying birth defects into subgroups that are etiologically and pathogenetically more homogeneous) and specified criteria for case inclusion; 3) an interview instrument which was developed collaboratively and administered at all sites obtains information on relevant exposures and potential confounders; 4) large sample size which provides unprecedented power to evaluate potential risk factors for specific birth defects; and 5) the use of biologic markers for exposure and susceptibility.

1.A. Summary of the National Birth Defects Prevention Study

In 1998, Congress passed legislation that directed the Centers for Disease Control and Prevention (CDC) to establish the Centers for Birth Defects Research and Prevention (CBDRP). The Birth Defects Prevention Act of 1997 was originally introduced in 1992 and passed in 1998 (Attachment 1). Money was appropriated in 1996 for CDC to initiate some of the activities described in the bill, which included the funding of the CBDRP.

In 1997, cooperative agreements for $800 thousand dollars per year for a period of five years were awarded to seven states (Attachment 2) to establish the CBDRP and support their collaboration in activities aimed at the prevention of birth defects. Specifically, these awards were designed to: 1) bolster ongoing surveillance activities (including the integration of prenatal diagnoses into surveillance registries); 2) develop, implement, and evaluate local studies (including research, special services, and program evaluation); and 3) contribute 400 interviews per year (300 case interviews and 100 control interviews) to the National Birth Defects Prevention Study (NBDPS). A competitive renewal process for additional 5-year cooperative agreements occurred in June of 2002. Two new Centers, North Carolina and Utah received funding as a result of this recompetition. The North Carolina and Utah Centers began data collection in the Fall of 2003. Data collection for the new Centers includes births occurring after December 31, 2002. Two Centers (New York and New Jersey) did not receive funding in June of 2002. New Jersey does not currently collect new data. The New York Center received full funding in September 2004 and began collecting new data. For fiscal year 2004, each funded center received approximately $900,000. Another competitive renewal process occurred in December 2008. All Centers receive approximately $1,000,000 for additional 5-year cooperative agreements.

The NBDPS is a case-control study of birth defects risk factors and is based on the existing birth defects surveillance registries in the nine active CBDRP. Interviews have been conducted with 32,662 women as of March 2009, including 23,738 mothers of infants with birth defects and 8,924 mothers of infants without birth defects.

1.B. History and Purpose of Birth Defects Risk Factor Surveillance

The Atlanta Birth Defects Risk Factor Surveillance Project (BDRFS), which was initiated in 1993, is a surveillance-based approach to evaluating risk factors for birth defects (CDC Protocol #1104, OMB #0920-0010). BDRFS was based on the Metropolitan Atlanta Congenital Defects Program (MACDP)(CDC Protocol #1955), which has been in existence since 1968. MACDP is a population-based, multiple source case ascertainment birth defects surveillance system for the five county metropolitan Atlanta area (Clayton, Cobb, Dekalb, Fulton, Gwinnett).

The Atlanta BDRFS studied selected MACDP case-infants and a random sample of control infants. The BDRFS protocol (CDC IRB #1104) which was in effect for births occurring between January 1, 1993 and early 1997, had three main components: 1) parental interviews; 2) improved birth defects classification; and 3) biologic specimens for use in evaluating biologic markers of exposure and susceptibility. Each year, maternal interviews were conducted with about 300 mothers of case-infants and 100 mothers of control-infants. In addition, biologic samples were requested of approximately half of these mother-infant pairs. (More than 400 biologic specimens from Atlanta BDRFS participants were banked).

The goals of the Atlanta BDRFS were to: 1) gain new information on causes of birth defects; 2) evaluate factors already suspected of influencing the occurrence of birth defects; 3) develop new surveillance methods; 4) maintain a biologic specimen bank that could be used in the future to generate and test hypotheses (evaluating biological markers of exposure and susceptibility risk factors for birth defects); and 5) develop and test methods in birth defects surveillance and research which could be exportable to other birth defects surveillance systems.

In 1993, CDC funded two five-year cooperative agreements with Iowa and California to conduct the BDRFS using their own surveillance systems. Among the three participating sites, 1,995 interviews were completed (1,213 case mothers and 782 control mothers). Several specific analyses have been published (e.g. obesity and birth defects, fertility treatment and craniosynostosis). Substantial experience was gained during the five year BDRFS effort. This experience provided a strong framework for the development of the NBDPS and the development of this revised protocol, which includes births occurring after October 1, 1997. The

BDRFS experience also provided experience for the oversight of NBDPS activities, the design of the computer assisted telephone interview, the development of tracking systems, and the development of a pooled relational database.

2. NBDPS INVESTIGATORS AND COLLABORATORS

2.A. CDC Investigators

2.A.1. Centers for Birth Defects Research and Prevention

This activity involves collaboration between the National Center on Birth Defects and Developmental Disabilities (NCBDDD) of the CDC, and the state-based CBDRP. Jennita Reefhuis, PhD, Birth Defects Epidemiology Team, is the Project Officer for the CBDRP and Tineka Yowe-Conley is the Study Coordinator. Dr. Reefhuis and Ms. Yowe-Conley are primarily responsible for the direction and administration of the CBDRP. As project officer, Dr. Reefhuis is responsible for directing and providing technical assistance to the CBDRP in the development of the NBDPS protocol, evaluating study conduct, and oversight of the individual cooperative agreements with the CBDRP. Dr. Reefhuis is responsible for insuring that all IRB and OMB requirements are met. In addition, Dr. Reefhuis is the lead scientific consultant to the NBDPS. Dr. Reefhuis has responsibilities for providing technical assistance to the CBDRP including study design, protocol development, data storage, and data management. As study coordinator, Ms. Yowe-Conley is responsible for the day-to-day management of the study and for coordinating activities among the Centers, and preparing and submitting all IRB, OMB and Certificate of Confidentiality applications.

Data Manager/Programmer, Chris Cosper and Programmer John Sims are responsible for security, transfer and maintenance of NBDPS-related data. They also design, program, and implement custom applications to assist in the execution of the study. In addition, they support,

instruct, and coordinate the data pooling efforts of CDC contractors and data managers from the nine CBDRP.

Mary Jenkins, PhD is responsible for coordinating the biologics component of the NBDPS. Dr. Jenkins is responsible for overseeing the collection, storage and analysis of biologic specimens for CDC and the NBDPS, including the submission of individual one page genetic research descriptions to the CDC Institutional Review Board (IRB) for review.

Stuart Shapira, MD is responsible for providing technical assistance related to case definition and birth defect classification and for clinical review of potential NBDPS participants.

2.A.2. CDC Site for the NBDPS

The CDC CBDRP in Atlanta, one of nine study sites, is an activity which involves the collaboration of NCBDDD and the Division of Laboratory Sciences (DLS) at the National Center for Environmental Health (NCEH). As Co-Principal Investigators of the Atlanta NBDPS, Dr. Reefhuis and Sarah Tinker, PhD are responsible for the study protocol, study conduct, interview instrument, and scientific aspects of the study design, data management, and analysis. In addition, Dr. Reefhuis and Dr. Tinker are responsible for meeting human subjects' requirements, supervising the activities of the CDC NBDPS staff, and collaborating with the other nine Centers for Birth Defects Research and Prevention.

Dr. Mary Jenkins is responsible for the collection, storage and analysis of biologic specimens for CDC and the submission of individual one-page genetic research descriptions to the CDC IRB for review. She is responsible for coordinating the efforts between the NCBDDD and the DLS at the NCEH. Dr. Shapira determines the eligibility of all of the cases included in the CDC NBDPS and collaborates with the clinicians at all of the CBDRP.

Dr. Jan Cragan has primary responsibility for MACDP and its related projects. Carolyn Sullivan and Marques Merriweather under contractual agreements with Abt Associates, Inc.

share responsibilities for record management and coordination of the Atlanta study, including case identification, and management of the study tracking system.

Dr. Peg Gallagher, DLS at NCEH, is the lead scientist responsible for assessment of the biologic specimens that are collected in the CDC NBDPS as well as for the Centralized Laboratory (biologic specimens from all Centers to be stored at the CDC and ATSDR Specimen Packaging, Inventory and Repository (CASPIR) facility). Cynthia Sturchio is responsible for the coordination activities of the Centralized Laboratory.

In addition to the above investigators, there may be a variety of other CDC investigators involved at any one time with this surveillance and research project. Some of these include:

i) The National Center on Birth Defects and Developmental Disabilities:

Margaret A. Honein, PhD, MS

Richard Olney, MD

Sonja Rasmussen, MD, MS

Adolfo Correa, MD, PhD

Suzanne Gilboa, PhD

Molly Cogswell, DrPH, RN

Cheryl Broussard, PhD

2. B. CBDRP Investigators

2.B.1. Arkansas

University of Arkansas for Medical Sciences
College of Medicine Birth Defects Research
Arkansas Children's Hospital Research Institute

Charlotte A. Hobbs, MD, PhD
Principal Investigator
University of Arkansas for Medical Sciences
Director and Professor of Pediatrics
13 Children's Way, Slot 512-40
Little Rock, AR 72202

2.B.2. California

March of Dimes, California Research Division
California's Hospital Oakland Research Institute

Suzan Carmichael, PhD
Co-Principal Investigator
March of Dimes/California Research Division
California's Hospital Oakland Research Institute
5700 Martin Luther King, Jr. Way
Oakland, CA 94609

Gary Shaw, DrPH
Co-Principal Investigator
Stanford University
Medical School Office Bldg
251 Campus Dr. Rm x159
Stanford, CA 94305-5460

2.B.3. Iowa

College of Public Health
The University of Iowa

Paul Romitti, PhD
Principal Investigator
Assistant Professor, Department of Epidemiology
Director, Iowa Birth Defects Registry
200 Hawkins Drive, C21-E GH
Iowa City, IA 52242

2.B.4. Massachusetts

Massachusetts Department of Public Health
Bureau of Family Health and Nutrition
Boston University Slone Epidemiology Unit
Brigham and Women's Hospital

Marlene Anderka, ScD, MPH
Principal Investigator
Massachusetts Dept of Public Health
250 Washington Street, 5th Floor
Boston, MA 02108-4619

2.B.5. North Carolina

University of North Carolina, Chapel Hill
School of Public Health
Department of Epidemiology
North Carolina Department of Health and Human Services

Andy Olshan, PhD
Co-Principal Investigator
Professor and Chair, Epidemiology
UNC School of Public Health
2104 E McGavran-Greenberg Hall
CB#7435
Chapel Hill, NC 27599-7435

Bob Meyer, PhD
Co-Principal Investigator
Director of NC Birth Defects Monitoring Program
1908 Mail Service Center
Cotton Building
Raleigh, NC 27699-1908

2.B.6. New Jersey

New Jersey Department of Health and Senior Services
New Jersey Department of Health and Senior Services
P.O. Box 364
Trenton, NJ 08625-0364

Leslie M. Beres, MS, CPM
Principal Investigator
Program Manager, Early Identification and Monitoring
Program Special Child Health and Early Intervention Services
Division of Family Health Services Public Health Services Branch

2.B.7. New York

New York Department of Health
Congenital Malformation Registry

Charlotte Druschel, MD, MPH
Principal Investigator
New York Department of Health
547 River Street, Room 200
Troy, NY 12180

2.B.8. Texas

Texas Department of State Health Services
Birth Defects Epidemiology and Surveillance Branch

Mark Canfield, PhD
Co-Principal Investigator
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756

Peter Langlois, PhD
Co-Principal Investigator
Texas Department of Health
1100 West 49th Street, T-707
Mail Code 1964
Austin, Texas 78756

2.B.9. Utah

University of Utah Health Sciences Center
Division of Medical Genetics

Marcia Feldkamp, PhD, PA, MSPH
Principal Investigator
Utah Birth Defects Network
P.O. Box 144699
Salt Lake City, UT 84114-4699

The principal investigators at each CBDRP work collaboratively with CDC scientists on scientific aspects of study design and analysis, including development of the study protocol, interview instrument design, and study conduct. In addition, they are responsible for: 1) meeting human subjects research and IRB requirements; 2) data storage and management; 3) clinical review of potential cases; 4) statistical aspects of study design and analysis; 5) collecting, processing, storing, and analyzing DNA from buccal swab specimens; and 6) coordinating a variety of laboratory components of the study. (This may include assessments of some biologic markers of exposure in selected participants, and/or the development and testing for possible environmental toxicants in biologic specimens.)

Collaborators at the Boston University Slone Epidemiology Unit have additional responsibilities, including provision of an up-to-date drug dictionary on a quarterly basis to be used for coding of reported prescription and nonprescription drug use.

2.C. Other Collaborators

In an effort to further understand birth defects risk factors, there may be a variety of additional investigators involved at any one time with this study. Such collaboration is essential to the success of this project because it allows scientists with differing expertise to work together, substantially improving the ability to better understand birth defects risk factors. Attachment 2 lists other researchers and project-related staff currently involved with the CBDRP.

In addition to the collaborators from the CBDRP, a number of individuals employed by Battelle/CPHRE and Abt Associates, Inc. will have important roles in this project. Battelle will be providing interview services for North Carolina and Abt Associates, Inc. will be providing interview services for Atlanta. These two organizations will have specific responsibilities, including: 1) maintaining the interview instrument; 2) tracing, contacting, and interviewing study subjects; 3) developing and updating study tracking systems; 4) providing monthly reports; 5) collecting biologic specimens; and 6) providing complete, clean, and edited data in a timely fashion. Abt Associates, Inc. will also provide the coding of interview data.

Primary Personnel:

Battelle Memorial Institute/Survey Research Operations

Charles Knott
Battelle/CPHRE
100 Capitola Drive, Suite 200
Durham, NC 27713-4411
(919) 544-3717

Abt Associate, Inc.

Gabriella Newes-Adeyi, PhD, MPH
Abt Associates, Inc.
4550 Montgomery Avenue, Suite 800 North
Bethesda, MD 20814-3343
(301) 634-1758

Deborah Klein Walker, EdD
Abt Associates, Inc.
55 Wheeler Street
Cambridge, MA 02138
(617) 349-2390

Rebecca Devlin
Abt SRBI
640 N LaSalle Street, Suite 640
Chicago, IL 60654
(312) 529-9705

Cathy Murphy
Abt Associates, Inc.
4620 Creekstone Dr, Suite 190
Durham, NC 27703
(919) 294-7752

3. METHODS AND MATERIALS

3.A. Description of State-Based Birth Defects Surveillance

All of the nine CBDRP have population-based birth defects surveillance systems (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New York, Texas and Utah) that have legislative authority to collect information on infants with major congenital malformations. A description of the surveillance system in each state can be found in Attachment 3 (A copy of the document providing this authority to the CDC is included in Attachment 3A). Each program monitors all births occurring to residents in a defined geographic area having at least 35,000 births each year. These birth defects surveillance programs include information on live born and stillborn infants diagnosed with at least one major birth defect within the first year of life, with diagnoses ascertained up to 5 years of life. Similar methods of multiple source case ascertainments are used at each site; most cases are registered through regular visits to local hospitals by members of the CBDRP surveillance staff, where records such as log books and patient's charts in nurseries, maternity units, and pediatric wards are reviewed to obtain clinical information and basic demographic data. Cases are also identified from the records of local cytogenetic laboratories, prenatal diagnosis clinics, genetic clinics, and vital records. Certificates of live birth, infant death, and stillbirth are supplied by state health departments.

Data are abstracted at each CBDRP onto a case record, which has been designed to meet specific surveillance needs at the site. However, all CBDRP case records include the same basic

demographic information, specific written diagnoses, 6-digit diagnostic codes, birth related information, cytogenetic data, complications of birth, prenatal data, pregnancy history, family history and other risk factor information.

The use of prenatal diagnosis for birth defects has become increasingly prevalent over the past decade. In many instances, because of a diagnosis during pregnancy of a serious birth defect or chromosomal abnormality, the pregnancy is electively terminated. For surveillance systems to have complete population-based ascertainment of birth defects, it is now necessary to obtain information from prenatal diagnosis clinics and on elective terminations of pregnancy. It is important to include prenatally diagnosed cases in any epidemiologic study of birth defects; without such inclusion criteria, an increasingly substantial number of case-infants will not be included in the study, which will likely make interpretation of study results very difficult. All CBDRP plan to include prenatally diagnosed cases, to the fullest extent possible.

3.B. The National Birth Defects Prevention Study Methods

The National Birth Defects Prevention Study (NBDPS) is a collaborative case-control study of birth defects risk factors, which is based on the existing birth defects surveillance registries of the nine CBDRP. The primary goal of this study is to improve the understanding of the causes of birth defects. It is anticipated that information obtained from this study is likely to be ultimately useful in the prevention of birth defects.

Using a computer assisted telephone interview (CATI), NBDPS interviews have been conducted with more than 35,644 women as of March 2010, including 25,957 mothers of infants with birth defects and 9,687 mothers of infants without birth defects. Because data (without personal identifiers) from all nine CBDRP is being pooled electronically for analysis (see sections 3.B.5. and 6.B), scientists within the CBDRP are committed to using a unified approach. It is critical to the overall success of the study to limit the introduction of site-specific differences, which may lead to biases, differential response rates, and other potential compromises to the overall quality of the data. To minimize data comparability problems, identical procedures and materials will be used to the fullest extent possible by all participating institutions. CBDRP scientists have been working closely together since the Fall of 1996 to

design and implement the NBDPS using a standardized study protocol. A letter from the CDC original project officer (Mr. Edmonds) stating the importance of identical study procedures was sent to all CBDRP and included in individual human study subjects submissions (Attachment 4).

Committees were formed with representatives from each CBDRP to design the collaborative case-control study. The **Coordinating Council** votes on all coordinating activities related to the NBDPS. The **standards committee** designed the protocol for the study including standard forms and procedures for identifying, contacting, and interviewing study participants. The **questionnaire and methods committee** revised the Birth Defects Risk Factor Surveillance (BDRFS) mother interview instrument and arranged to have the questionnaire placed into a CATI format. The **clinicians committee** (geneticists and clinicians from each CBDRP) decided on the case definitions for the 30 birth defects categories included in the study and developed guidelines to assist with case identification and review, medical record abstraction, and coding. The **biologic committee** designed the protocol for the collection of biologic and environmental samples and developed a plan for banking and sharing the biologic specimens. The **data sharing committee** (Section 3.B.5.a) has the ongoing task of deciding how the data will be equitably shared for analysis. This committee is responsible for review of all protocols for data analysis as well as addressing human subjects' issues, data access, collaboration, and authorship.

The efforts of these committees and the efforts of the CBDRP scientists are described in more detail on the following pages of the NBDPS Protocol. As further described in this document, each site proposes to use to the fullest extent possible, the following identical approaches:

1) case definition - (Sections 3.B.1. and 4.A., Attachments 5 and 6)

2) control selection - (Sections 3.B.2. and 4.B)

3) letters of introduction - (Sections 4.C.1 and 4.C.2, and Attachment 18)

4) tracing procedures - (Section 4.C. and Attachment 15)

5) contacting procedures - (Sections 4.C. and 4.D., Attachment 22 and 23)

6) telephone interview - (Section 3.B.3 and Attachment 8 and 22)

7) informed consent - (oral and written) (Section 5, Attachment 10 and 22)

8) collection, processing and storing of biologic samples -
(Sections 3.B.4. and 6.D., Attachments 9 and 25)

9) calculation of participation rates (Section 3.B.5.c., Attachment 14)

10) replication, editing and use of data (Sections 3.B.5, 6.B., and 6.C).

3.B.1. Case Definition

Clinicians at each CBDRP worked together to develop the NBDPS case definition. Infants are eligible for inclusion if they have one or more defects from the list of selected birth defects included in Attachment 5. In addition to selecting birth defects, which are of unknown or uncertain etiology, these defects were selected for one or more of the following reasons. The defect is:

a) considered to be a major defect (affecting survival, requiring substantial medical care, or resulting in marked physiological or psychological impairment);

b) usually identifiable in the first 6 weeks of life (may be extended for some defects); and

c) consistently classifiable.

In addition, other criteria may be considered, including:

a) the defect is either common (and thus of public health importance) or rare (and thus unlikely to be studied in smaller studies);

b) the pathogenetic mechanism of the specific defect is similar to other included defects; or

c) there are specific etiologic hypothesis(es) which require additional study.

Cases can be: 1) live born infants; 2) stillborn infants greater than 20 weeks gestational age or 500 grams; or 3) prenatally diagnosed and terminated fetuses at any gestational age or weight.

3.B.1.a. Clinical Review

Numerous studies have documented extensive etiologic heterogeneity in birth defect cases with similar anatomic problems. For example, neural tube defect cases that have no associated defects have different epidemiologic characteristics and familial recurrence risk patterns from cases that have other defects. To provide a sound epidemiologic framework to

study specific defects, the presence of associated defects, and accurate clinical descriptions of defect types can and should be used in classifying birth defect cases into subgroups that are etiologically and pathogenetically homogeneous.

To accomplish this goal, clinical staff reviews the abstracted medical records of case-infants which are ascertained each year by the individual CBDRP to determine if that case-infant meets the specified case definition and inclusion criteria. The clinicians use a standard clinical review and classification protocol, which was developed by CBDRP clinicians (Attachment 6) working closely together. To evaluate case eligibility, the clinicians use a system of case-notes in the clinical database shared between CBDRP to have questions and issues rapidly resolved. Phone conferences and meetings are also scheduled as needed.

General inclusion and exclusion criteria include:

1) certain types of birth defects cases which have been ascertained solely through prenatal diagnosis will be included (including the method of diagnosis, as noted by clinical reviewer);
2) cardiac defects will be included if the diagnosis is based on echocardiography (at least);
3) cases with the following known etiology are excluded: chromosomal/micro deletion disorders and single gene disorders; and
4) cases with teratogenic syndromes and recognized phenotypes of unknown or uncertain etiology are included.

3.B.1.b. Clinical Database

A clinical database which has been developed by the collaborating clinicians and the Programmers/Data Manager (Attachment 7) provides additional detailed information to aid in classification of cases during analysis. The information contained in the clinical database is obtained through the abstraction of medical records. The database includes physical attributes of the infant (e.g. weight and head circumference), verbatim diagnoses (from medical records) of birth defects, exams used to determine diagnoses, relevant cytogenetic/molecular tests, family history information, date of death, cause of death, and autopsy results, if available. The clinical

databases from each CBDRP are compiled at CDC without personal identifiers and linked by study identification number (ID) to the interview database. The clinicians also review each case and classify the infant into appropriate categories for analysis. For example, infants may have one major defect (isolated case) or 2 or more major birth defects in different systems (multiple case).

3.B.2. Control Selection (See also Section 4.B.)

Control-infants from each CBDRP will be selected randomly either from vital records (birth certificates) or from hospitals of birth. Using birth certificates to identify controls is only an option in states where vital records are recorded electronically in a timely manner (generally within weeks of delivery). The CBDRP which use birth certificates for the selection of controls are:

1) Iowa: access to these certificates is obtained through a signed research agreement with the Iowa Dept of Public Health;
2) Massachusetts: access to these certificates is obtained through Massachusetts 24A and 24B review board and departmental IRB approval;
3) New Jersey: authority, through the Health Department, was to select controls from the Electronic Birth Certificate system; controls are no longer selected.
4) Georgia: as an agent for the Georgia Dept. of Human Resources, the Centers for Disease Control and Prevention has legal authority for the collection of health information, as provided in Chapter 12 of the Official Code of Georgia (OCGA). With this authority, CDC routinely reviews medical records of births in the five-county metropolitan Atlanta area to determine if birth defects are present and to abstract information, as necessary to conduct the Metropolitan Atlanta Congenital Defects Program. In addition to having this authority, the protocol for selecting controls in this manner has been in place since the beginning of this study in 1993 (CDC protocol #1104). Original IRB approval of this method was granted on May 14, 1992 and the most recent approval of protocol #1104 was granted on March 2, 2009 (exp. March 9, 2010). Beginning with births in January

2000, we began selecting some controls from vital records, and beginning with 2001 births all controls has been selected from vital records.

5) North Carolina: authority, through the NC Birth Defects Monitoring Program within the NC DHHS, is to sample from birth certificates and take personal identifiers.
6) Utah: authority, through the Health Department, is to select controls from the Electronic Birth Certificate system.

The CBDRP which use hospital data for the selection of controls are:

1) New York: uses the CDC's control selection protocol to select controls from hospital birth records; the Health Department Commissioner or his/her agents has statutory authority to carry out studies;
2) California: has a legal mandate to obtain information on infants without malformations to serve as controls;
3) Arkansas: uses the CDC's control selection protocol to select controls from hospital birth records. The authority to do this was established through a legislative act in 1985. This legislation states that the purpose of Arkansas Reproductive Health Medical System (ARHMS) is to "collect and analyze data from a number of sources to describe trends in reproductive endpoints". All hospitals with patient records containing information pertaining to reproduction and development are required to share information in those records with the ARHMS.
4) Texas: uses the CDC's control selection protocol to select controls from hospital birth records. Texas has a state law that allows them to get controls from hospitals.

In anticipation of a 70% participation rate (based on the experience from the BDRFS study), each CBDRP selects randomly from the population (from either vital records or hospital birth logs) approximately 150 eligible controls each year for inclusion in the study. A randomly selected birth is not eligible for inclusion in the study if the chosen infant:

1) is actually a case or has major birth defects ascertained within one of the CBDRP birth defect surveillance systems;
2) is not a resident of the geographic area covered by one of the CBDRP population-based registries at the time of delivery;
3) is adopted or in foster care;
4) has a deceased mother; or
5) is a stillborn.

Whether hospital records or birth certificates are used as the source for control-infants, the records are reviewed to insure the selected birth is not a case-infant and to abstract information for the purpose of follow up and contact.

3.B.3. Interview Instrument

Mothers of all case and control infants who agree to participate in the NBDPS are interviewed by telephone in a search for birth defects risk factors. This interview provides the framework for the NBDPS, providing critical information, which is used in all aspects of the study. Building on interviews of over 2,500 mothers of infants with and without birth defects from the original BDRFS study (OMB 0920-0010; expires 3/31/2012), a one-hour computer assisted telephone interview (CATI) was programmed for the NBDPS (Attachment 8).

In summary, the BDRFS telephone interview was modified by: 1) updating the instrument to evaluate possible new and emerging birth defects risk factors (e.g. new prescription and nonprescription drugs, diet); 2) rewording some questions to improve the quality of exposure information obtained; 3) deleting some questions and sections which proved to be less fruitful than originally expected; and 4) expanding other sections to provide necessary increased detail.

The NBDPS interview instrument contains sections on pregnancy history (including prenatal diagnosis), maternal conditions and illnesses, family history, lifestyle and behavioral factors (including alcohol use and substance abuse), nutrition, multivitamin use, environmental exposures, occupational history and physical activity.

The interview instrument underwent an evaluation in 2003, and a revised interview instrument was approved in 2005. The order of the questionnaire was changed to make the flow

more comfortable for participants. We also added a few questions on maternal stress, dieting, diarrhea, and paternal smoking. We shortened the sections on drinking water, illicit drug use, occupation, and pregnancy to keep the total questionnaire length approximately the same. The revised CATI was implemented for births beginning January 1, 2006.

More recently, the NBDPS Nutrition Work Group proposed adding seven questions to provide information about the prevalence of periconceptional physical activity and association of physical activity and major birth defects. These seven questions are taken from the International Physical Activity Questionnaire (IPAQ). The full IPAQ assesses all domains of physical activity. We have slightly modified the seven IPAQ questions which focus on usual physical activities to assess the timeframe "during the 3 months before you became pregnant" because our objective is to measure periconceptional activity and we assume that physical activity changes little over the three months before pregnancy through the end of the first trimester. This version of the IPAQ was pilot tested among three CDC volunteer staff members who were between 6 and 24 months postpartum. The volunteers were asked about the clarity, comfort, wording and overall assessment. Average administration time was four minutes. Therefore, these additional physical activity questions will add four additional minutes to the current interview which is estimated to take one hour. The placement of this new section is not in alphabetical order and was purposely arranged this way to avoid relabeling the current questions and to ensure the additional questions do not affect the responses to other questions. The questions are placed in SECTION P: Physical Activity (pages 79-81), between SECTION I: Father's Occupation and SECTION J: Family's Demographic of the questionnaire.

The primary language of the NBDPS interview instrument is English. However, the interview has been translated to Spanish, and 12% of interviews have been completed in Spanish. In addition, letters of correspondence, telephone scripts and consent forms have been translated for Spanish speaking participants.

3.B.4. Biologic and Environmental Samples

3.B.4.a. Background

Although interview instruments are a major tool in the search for causes of birth defects, they have limitations for evaluating genetic susceptibilities to disease and limitations for evaluating certain exposures because of problems of recognition and recall. In a concentrated effort to improve our understanding of the etiology of birth defects, particularly in the area of gene-environment interactions, we are collecting biologic samples for use in the evaluation of biologic markers of exposure and susceptibility.

Collaborating CBDRP scientists are collecting cheek cell samples (buccal brushes) on all case-infants and control-infants, and their parents. The CBDRP researchers bank specimens collected as part of the NBDPS, storing the samples in a manner which will permit efficient retrieval and optimum stability for later use in studies related to birth defects etiology. (See Section 3.B.4.c.)

In general, use of banks for the storage of biologic specimens is becoming increasingly important for epidemiologic research for several reasons. Major expenditures in time and money are spent in sample collection. The ability to use banked specimens to test new hypotheses or to utilize new techniques is advantageous to the research efforts to understand the causes of birth defects. In addition, by maintaining biologic specimen banks, which have the capacity to allow for research tests as new hypotheses or improved technologies emerge, the potential contributions of study participants are maximized. This approach is the most reasonable, provided participants are informed of the intent to bank their specimens and the intent to use their specimens for such research studies.

The use of a biologic specimen bank that can be built over time and maintained indefinitely is particularly important for testing hypotheses regarding risk factors for birth defects. Serious birth defects occur in about three percent of all births; individual defects are much rarer (incidence of individual defects ranges from approximately 1 in 1000 live births to 1 in 10,000 live births). Because of the rarity of individual defects, many years of data collection are required to obtain enough cases of a specific type of birth defect to complete a particular

etiologic study. The length of time between obtaining the biologic specimen and the availability of adequate numbers for a specific analysis is likely to be a minimum of two to five years, and may well be much longer; the banking of biologic specimens, therefore, is a lengthy process.

Of note: In previous CDC IRB reviews of the Atlanta BDRFS, there has been much discussion surrounding issues related to genetics research. This protocol incorporates the approaches proposed by the NBDPS and approved by the CDC IRB in previous reviews of the Atlanta Birth Defects Risk Factor Surveillance Project (CDC Protocol #1104).

3.B.4.b. Collection and Use of Buccal Cells for NBDPS Participants

The collection of buccal cells provides a relatively simple, inexpensive, and convenient means of obtaining DNA samples for use in evaluations of genetic differences at specific gene loci. Buccal cell samples are collected on a sterile brush by rotating it on the inner cheek. Because the procedure is simple and noninvasive, participants can collect the samples, using materials sent to them at home, and return the samples by mail.

Scientists with the CBDRP collect cheek cell specimens from NBDPS participants to be used to analyze DNA markers. Each CBDRP collects cheek brushes from each case and control infant and their mother and father.

3.B.4.b.1 ORAgene-250 Saliva Pilot Study

As previously mentioned, DNA samples are currently self-collected from participants of the National Birth Defects Prevention Study (NBDPS) using two cytobrushes. Lower yields and lesser quality DNA has been reported when it is derived from buccal cells collected using cytobrushes compared to DNA derived from saliva. However, the literature is not clear on whether saliva-derived DNA from infants or toddlers is of higher quantity and better quality than cytobrush-derived DNA. The purpose of this pilot study is to compare the two collection methods in the same infant or toddler.

We enrolled up to 25 infants or toddlers, ages 7 months to 19 months old. We asked the mothers to use the ORAgene-250 kit with a package of 5 sponges to collect saliva from her child(ren). When saliva collection was completed and the kit was returned to us, we sent her a kit that contains 2 cytobrushes to collect buccal cells from the inside cheeks of her child(ren).

The completed Oragene-250 saliva kits were sent to Dr. Richard Finnell's lab in Texas (an NBDPS collaborator). The Finnell lab staff extracted DNA from the saliva; they are proficient in DNA extraction from Oragene-250 kits. They sent the DNA to the NBDPS Central lab. The NBDPS Central lab staff extracted DNA from cytobrushes; they are proficient in DNA extraction from cytobrushes. They also measured human-specific DNA in cytobrush-derived and saliva-derived samples using an RNAseP assay followed by microsatellite analysis to assess DNA quality. The DNA samples will be destroyed within 3 months of analysis. The results of the pilot study were obtained in February 2009. The decision to switch to saliva collection is pending further discussion by the CBDRP Coordinating Council.

3.B.4.c. Banking of Biologic Samples

After the cheek cell samples have been collected, each CBDRP retains at their site, one brush. The other brush is sent to the CDC Centralized Laboratory for processing. Once processing is complete at the Centralized Laboratory, samples are sent to the CDC storage facility (CASPIR) where they will be stored and identified only by study ID number.

It is the expectation of scientists within the CBDRP that a portion of the DNA will be banked for very long-term research studies, perhaps even decades in the future, when the technologies available are likely to be able to make use of these in ways that can only be imagined now (perhaps, for example, by carrying out sequencing of the entire human genome in each individual sample). These samples will be stored indefinitely unless a request is received from the participant to destroy them.

CBDRP scientists plan to share aliquots of these samples, without personal identifiers, to carry out collaborative research studies, as approved by appropriate internal and external review of the proposed research. (Attachment12). Quality control DNA studies have begun among the CBDRP and a protocol for how the samples are made available to CBDRP scientists has been developed. As previously mentioned, sharing of samples with collaborating investigators is done without personal identifiers, unless specific permission has been obtained from human subjects committees at the participating institutions. Only researchers within the CBDRP will have access

to these materials. Investigators retain control of biological materials obtained at their CBDRP, unless the participant requests that these materials be destroyed (Attachment 10).

Of note: There is no commercial value in these samples and profits from any materials associated with this study are not expected. The samples will not be used for commercial purposes. Neither researchers nor study participants will receive profits from the donated materials.

3.B.4.d. Evaluation of Genetic Susceptibilities to Birth Defects

Specimens stored in the NBDPS biologic specimen bank will be used to evaluate genetic susceptibilities to birth defects using candidate genes, genome wide associations, copy number variants, epigenetics, and other emerging technologies. Genes that are thought to play a role in the normal embryology and pathophysiology of different organ systems (e.g. growth factor genes, steroid receptors, and homeobox genes) will be studied; genetic variation in some of these genes may be involved in the pathogenesis of birth defects.

Increasing numbers of genes are being mapped and sequenced, and with the use of polymerase chain reaction (PCR) and other molecular technologies, it is becoming increasingly possible to evaluate the role of genetic differences at specific gene loci and their interaction with specific exposures in the etiology of birth defects. Molecular DNA technology is moving at a tremendously fast pace, and more potential genes and genetic markers are available almost daily. Therefore, it is difficult, if not impossible, to know which new genes or genetic markers will be available for study even in the very near future, or which of the available genes would be selected in our research efforts to better understand birth defects risk factors; individual research tests may or may not be performed on specific samples.

For each of the major classes of congenital anomalies, a number of candidate genes warrant further research. Because of the known association between periconceptional folate supplementation and prevention of neural tube defects, genes involved in folate metabolism, such as the methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase genes, as well as the alpha and beta folate receptors, will be studied. Since micro deletion of the chromosome 22q11.2 region has been identified in some cardiac defects, microdeletion studies

using DNA markers from this region are of interest in the study of congenital heart defects. Studies on clefting conditions might include a number of candidate genes thought to potentially play a role in the causality of cleft palate, including transforming growth factor alpha (*TGFa*), transforming growth factor beta 3 (*TGFb3*), retinoic acid receptor (*RARA*), the proto-oncogene *BCL3*, and the thyroid hormone receptor alpha 1 (*THRA1*). For congenital anomalies involving the eyes, genes such as crystallin (associated with cataracts), the *PAX6* gene (associated with aniridia) and the *PITX2* gene (involved with Rieger syndrome, a multi system condition which includes iris abnormality as a feature) might be studied. Limb abnormalities could be studied with the *HOX-D13* and several T-box transcription factor genes that have been associated with limb abnormalities. These examples emphasize the broad range of different genes that might confer increased susceptibility to a particular birth defect and that may be included in our research study,

In addition, a number of disorders have been recognized as being associated with very specific gene mutations, and although not a primary goal of this project, research studies of these could make significant contributions to the range of mutations seen in those disorders. Examples include sonic hedgehog, associated with holoprosencephaly; the craniosynostosis syndromes associated with mutations in *FGFR1*, *FGFR2* and *FGFR3*; achondroplasia and the *FGFR3* mutation; Stickler syndrome and mutations in *COL2A1* and likely others. In addition to these evaluations, several other genetic factors have already become established in existing birth defect literature as focal points of biomarker research. Other candidate genes that could be explored include the GSTM1-null mutation and variants of other carcinogen-metabolizing genes such as cytochrome P450 (CYP) mixed function oxidases (e.g. *CYP1A1*, *1A2*, *2E1*).

It is apparent that the number of potential genes of interest is quite long and differs depending on the specific birth defect to be studied. In addition, many of the genes or genetic markers of potential interest have yet to be identified. Because it is not possible at this time to specify all genes to be studied in our quest to better understand birth defects, we plan to approach the human subjects committee with individual one-page research plans for each gene or genetic marker to be studied in our specimens. A brief proposal (one to two pages) providing

information on the specific gene/gene marker to be studied will be submitted (Attachment 11); the proposal will include justification for its study, potential clinical relevance of the information, and plans to deal with clinically significant findings (if any). A list of approved proposed genes for study can be found in Attachment 12.

3.B.4.e. Evaluation of Laboratories Genotyping NBDPS Specimens

To ensure that each lab actively involved in genotyping NBDPS specimens is proficient in their respective genotyping techniques independent of the source material or extraction procedure, a two-part annual external quality assessment (EQA) was developed.

The first part includes evaluation of a set of control DNA samples obtained from buccal cells and blood collected from mother, father, and child trios as part of a study approved by the University of Washington's (UW) IRB ("Genetic Analysis of Heart Defects"). DNA samples are de-identified and only the local research team at UW has access to the link to individual identities. Informed consent was obtained prior to specimen collection. Paired DNA specimens from blood and buccal cells from each control along with negative controls are distributed to each lab actively genotyping DNA samples collected for the NBDPS. Lab personnel are blinded to the source of each DNA specimen. Each lab handles the samples as they would typical NBDPS samples. If they plan to perform whole genome amplification (WGA) on NBDPS samples, they will do so on the blood-buccal trios and negative controls. Each lab will use their standard methodology to genotype the samples for a small number of gene variants agreed upon by the NBDPS Genetic Analysis Working Group. Genotyping results from 2-5 gene variants will be sent to the CDC each year to evaluate the following:

- Intra-lab comparison of results from blood compared to buccals and to WGA products
- Intra-lab verification of genotype accuracy by Mendelian inheritance
- Inter-lab comparison for results of one SNP each lab will genotype
- Inter-lab comparison of SNPs labs assay in common when possible

The second part of the external quality assessment (EQA) protocol includes purchasing a panel of multi-ethnic DNA samples from the Human Variation Collections of the National Institute of General Medical Science Repository. They have developed a resource of DNA

samples from unrelated individuals, male and females, designed to reflect the diversity in the human population (Polymorphism Discovery Resource). No medical, phenotypic, or ethnicity information will be associated with individual samples. The DNA samples came from individuals enrolled in other studies who gave informed consent explicitly to be part of these human variation panels. The DNA and negative controls will be distributed directly by The Coriell Institute for Medical Research. When labs receive the samples, they will genotype the genomic DNA using their standard genotyping methodology for a small number of variants agreed upon by the NBDPS Genetic Analysis Working Group. Lab personnel are blinded to the source of each DNA specimen. Genotyping results form 2-5 gene variants will be sent to the CDC each year to evaluate the following:

- Comparison to published third-party results
- Inter-lab comparison of SNPs labs assay in common

The standards required to pass EQA include the following:

- 90% genotyping call rate per gene variant
- 99% concordance between successful genotyping data for:
    - paired blood and buccal DNA
    - gDNA and WGA product
    - inter-lab SNPs assayed in common
    - pre-characterized DNA and published third-party results
- No results reported for negative controls
- Genotyping results of trios consistent with Mendelian inheritance

If inter-lab results for SNP assays performed in common are discordant, results from SNP assays performed on pre-characterized samples will be compared to third party published results to determine if a lab needs to identify and resolve problems. If a genotyping lab does not pass EQA standards, they must discontinue all genotyping and repeat EQA. If the genotyping lab does not pass EQA standards a second time, no manuscripts will be completed until the problems are identified and resolved.

3.B.4.f. Biologic Markers of Exposure to Environmental Teratogens

In addition to maintaining a biologic specimen bank for the purposes of conducting research to evaluate genes, it is important to maintain a specimen bank to allow better laboratory identification of possible teratogenic exposures, as new techniques become available.

Biologic markers of exposure are useful in reducing the effects of both differential and nondifferential exposure misclassification in epidemiologic studies. Differential misclassification occurs when the ability to recall exposure events varies among subgroups, a classic problem in the epidemiologic study of birth defects. Such misclassification, which is primarily due to recall bias, can lead to spurious associations between putative exposures and birth defects.

Nondifferential misclassification occurs when the proxy for exposure is imperfect but uniform across subgroups (case and control subjects). Because misclassification due to poor proxy measures (e.g., job titles) can jeopardize the epidemiologist's ability to detect true underlying risks, such nondifferential misclassification may play a substantial role in limiting the progress towards understanding birth defects etiology. In addition, biomarkers of exposure can be useful in quantifying exposure levels, which is an important epidemiologic tool when assessing dose-response relationships.

Because cheek cell samples are designed for the analysis of DNA only and not well suited for the evaluation of environmental exposures, other biologic samples would be required for this purpose. Although not included at this time in the general NBDPS Protocol, CBDRP scientists are evaluating and considering the use of other biologic samples such as blood, urine or saliva, to be used in evaluating environmental factors and gene-environment interactions.

3.B.4.g. Environmental Sampling

Some CBDRP investigators plan to use environmental sampling to quantify residential exposures, as appropriate and feasible. As an example, levels of DBP in tap water and in biologic samples (blood and urine) were evaluated in a small subgroup of these participants as a local study (1).

Because laboratory refinements in exposure assessment are occurring rapidly, having adequately collected and stored biologic samples for future evaluation is important. An example

of how new laboratory techniques will be helpful in the study of birth defects, is the use of a high-resolution magnetic sector spectrometer to evaluate individual volatile organic chemicals in blood. Using this instrument, scientists in EHLS have developed a unique analytical method that enables the determination of parts per trillion levels of 31 volatile organic chemicals (including DBP) in 10 mL of blood. This technique has been used in one local study (2).

3.B.4.h. Centralized Geocoding of NBDPS Residence Data

During the interview portion of the study, information is collected on residence addresses from three months before conception to date of index birth. Geocoding this information (i.e. assigning geographic coordinates) will be extremely valuable for studies of environmental health as well as other topics. For example, geocoding would allow studies of distance to points such as factories, toxic waste dumps, nuclear power plants, and health care facilities. It would also allow studies that require assigning residences to areas such as aquifers, geological regions (e.g. high radon areas), areas of water treatment utilities, plumes from pollution sources, census tracts and their variety of socioeconomic variables. The surveillance systems of some of the Centers are already geocoding case-infants as part of their routine surveillance of the patterns of birth defects occurrence.

The NBDPS plans to conduct geocoding for all interviewed cases and controls from all Centers for Birth Defects Research and Prevention, and some Centers have already begun this process on their local data. However, we have started to centralize the geocoding for all Centers to improve the consistency of coding across centers, and ensure that all data from all centers can be coded. Centralized geocoding will result in:

Increased consistency and quality control: Although there are several automated techniques for geocoding, it is expected that their accuracy will vary within and between Centers (e.g. accuracy is highest in urban areas). Also, a certain percentage of addresses for each Center will require interactive (manual) geocoding; one group doing that will maximize consistency across Centers.

No delays in coding data from a particular center due to lack of resources: While all Centers have the capability to geocode their NBDPS data (in-house or through contract), five Centers have stated they will need additional funding to implement it.

The first geocoding files were completed by ATSDR and made available for each Center on October 14, 2008.

Current IRB approval for each Center states that in order to protect confidentiality of subjects, no identifying information will leave the Center as part of routine NBDPS data collection. Thus the residential history data are not included in the monthly CATI replication to CDC. Some Centers have stated that even if data are geocoded centrally, all the geocoded and original address data must be returned to them. After the centralized geocoding is complete, all geocoded data will be returned to the center of origin; a centralized repository of the geocodes will NOT be maintained at CDC.

The Geospatial Research, Analysis, and Services Program of ATSDR have offered to geocode all NBDPS residence data from all Centers, at no cost to the NBDPS. This group, external to the NBDPS, does not need any information about the NBDPS participants besides the actual address. They will only know that at that address a child was born in the past 7 years, and that the mother participated in the NBDPS interview.

Objective

The purpose of this project is to complete centralized geocoding while maintaining subject confidentiality.

Procedures for Geocoding

Each Center obtained IRB approval from their institutions.

1. Each Center obtained residence data from their data manager for all cases and controls with estimated dates of delivery from the start of the study (births after 10/1/1997) up to the designated cutoff date for each data batch. For example, the first data batch included study IDs with a date of delivery of 10/1/1997 through 12/31/2003. This included all maternal residential addresses from 3 months before conception through date of delivery.

CDC IRB approval was received April 25, 2007 to expand geocoding of NBDPS address data to include all future data releases.

2. Each Center will locally clean the data (make sure spelling is correct for cities, etc.).
3. Each Center will replace the NBDPS identification number with a new ID number (per instructions, which will be independent of year of birth or case/control status), and must save the key relating the two numbers.
4. Each Center will send the cleaned residence history data without the original NBDPS identification number to a CDC/NBDPS contact person.
5. The CDC/NBDPS contact person will batch the data from all 10 Centers and send to the CDC/ATSDR geocoders.
6. The CDC geocoders will complete the geocoding and return the data with the geocodes to the CDC/NBDPS contact person.
7. The CDC/NBDPS contact person will split the data by Center and send to each appropriate Center.
8. Each Center will link back to the NBDPS identifier.
9. No data will be retained by the NBDPS team or the CDC/ATSDR team.

For each specific project, exposure assessment may be done locally according to precise instructions or may be sent to a central location for more consistent results. If the latter, the project will be sent for approval to the CDC IRB and the local Center IRB. The new ID number will again be used in order to protect confidentiality and to blind the exposure assessment personnel regarding case/control status.

CDC and local IRB approval were received to 1) share the geocoded data with the Centers doing the exposure assessment; 2) analyze the geocoded data from all Centers; and link the geocoded data to other data sources to explore a variety of environmental exposures.

The linkage of geocoded data to other data sources available to our researchers will provide important opportunities to examine numerous "environmental exposures." Some exposures of interest include pesticides, water contaminants, and air pollutants and their association with the development of birth defects. These research goals are in line with the scientific themes of the overall project.

In some instances, investigations that include geocodes will be led by collaborators from other Centers (within the multi-centered National Birth Defects Prevention Study). When an NBDPS investigator outside the CDC takes the lead on such a project, we propose several requirements that must be met in order to obtain geocoded data:

1. An investigator from the CDC will be a co-investigator on the project;
2. All collaborators must sign a certificate of confidentiality oath;
3. Only geocoded data relevant to the aims of the investigation will be made available to a collaborator;
4. The transfer of geocoded data will be done over a secure data network;
5. Collaborators must demonstrate and ensure the security of their data systems that will be used to maintain and analyze geocoded data; and
6. All geocoded data sent to a collaborator must be destroyed or returned to the upon project completion.

This process maintains the confidentiality of our data while allowing for collaborations that advance the goals of the project and provide new insights into the effect of environmental exposures on the development of birth defects.

The first exposure assessment project is currently being planned by the Iowa Center. On May 12, 2009, Iowa received approval to lead our multi-center efforts to link the geocodes for maternal residence with the public water utility (if applicable) that serves each residence. Upon IRB approval at each Center, utility data and geocoded data will be provided to the University of

Iowa Center for Health Effects of Environment Contamination (CHEEC). Investigators with CHEEC will map the geocoded data to public water utilities using GIS software to determine whether the maternal residence is served by the public water utility. Once the geocoded residence data have been linked to the appropriate public water utility, geocoded residence data will be returned to the respective Center. Only the NBDPS ID number and linked public water utility code for each mother will be retained for analysis.

Safe Drinking Water Act (SDWA) data for contaminants and time periods of interest available from public water utilities will be linked to each NBDPS ID number. The Iowa Center will link SDWA data for disinfection-by-products (DBPs), specifically trihalomethanes (THMs) and haloacetic acids (HAAs) and each NBDPS ID number. These data will be sent to the North Carolina Center for the NBDPS for temporal and spatial variablity assessment. Following this assessment, the data will be returned to the Iowa Center and linked to interview data on maternal water use and consumption. This linked dataset will be used by NBDPS investigators to examine the association between perioconceptional exposures and selected, major birth defects. The potential association between maternal periconceptional exposure to THMs and HAAs and orafacial clefts (OFCs) will be examined. (Iowa's project details and approval are attached)

Protection of Human Subjects

Geocoding staff would have access to address data of NBDPS subjects, but be unable to link with NBDPS data to determine case/control status or any other information. They will sign and be bound by the NBDPS confidentiality and data use oath.

3.B.4.i. Evaluation of Gene-Environment Interactions

Individual susceptibility (biomarkers of susceptibility) to the effects of environmental agents may vary depending on specific genetic or other host factors (e.g., nutrition or immune function). If the effect of an exposure on the occurrence of birth defects depends on the interaction between

the exposure and genetic susceptibility, then neglecting to study such interaction may lead to underestimating the magnitude of the association between the exposure and the outcome.

As previously mentioned (Section 3.B.4.d.), it is increasingly possible to evaluate the role of genetic differences at specific gene loci and their interaction with specific exposures in the etiology of birth defects. Candidate genes that could be explored include the *GSTM1*-null mutation and variants of other carcinogen-metabolizing genes such as cytochrome P450 mixed function oxidases. Together, these genetic variants of enzymes involved in detoxification reactions may play a significant role in the metabolism of DBP in tap water and other environmental exposures. Since evidence suggests that these genetic variants may modify susceptibility to a range of adverse health effects, polymorphisms within these genes should be studied to evaluate their possible link with birth defects. One example is the previously mentioned *MTHFR* gene, in which evidence suggests that genetic polymorphisms within *MTHFR* can combine with environmental exposures to place a particular subpopulation at greater risk for having children with neural tube defects.

3.B.4.j. Future Access to Genetic Information

We intend to inform study participants of general study progress and research findings, as studies are completed. For this purpose, a roster of participants is being maintained and updated periodically. The first participant newsletter was mailed to participants in December 2000. Additional newsletters that describe the status and completed work of studies using the NBDPS are mailed to past participants on approximately a yearly basis. Because New Jersey did not receive funding in June 2002, the CDC CBDRP now receives only the names and contact information for the New Jersey participants. This is necessary to comply with a consent form (Attachment 10) previously signed by the participants which states "For any tests that have clinical importance, we will publish summarized results in the study newsletter. This newsletter is sent to all participants". The former New Jersey Principal Investigator mailed a final letter

informing their participants that New Jersey's contributions to the CBDRP are complete. A recent NBDPS newsletter was also included and the participants were advised to either call or email their contact information to the CDC if they wish to continue to receive an annual NBDPS newsletter. The CDC CBDRP only contacts and sends newsletters to those participants requesting to receive one.

We do not intend to provide participants with individual study results. However, for a limited period we allowed subjects to request their own results, if desired, for any clinically significant tests. The consent form was revised to clarify this as follows: studies that will be done on the collected biologic samples are not meant to test medical status. Since all studies will be done in research labs, there is no plan to notify participants of study results. Research labs do not have the same quality control standards as clinical labs. Research labs may also use less expensive techniques, which can make the tests less reliable than those from a clinical lab. However, a few of these studies may have clinical importance. For any tests that have clinical importance, summarized results will be published in a peer-reviewed journal and also the study newsletter. Participants are advised to contact their healthcare professional if they have any questions about whether or not genetic tests could be useful.

If NCBDDD receives a request for the results of individual genetic tests carried out in the NBDPS, we will comply with the Privacy Act and respond in the following way:

1. Most of the cheek cell samples will be stored for future use. If someone requests genetic test results and we have not done anything with their sample yet, we will inform them that their sample has not yet been included in any studies of clinical significance. We will reiterate what was included in their written consent about the cheek cells being used for future research and that results from these studies are not meant to test individual medical status, and that only results from tests that have clinical significance can be reported. We will tell them that if they are concerned

about genetic factors that may be associated with birth defects, we suggest that they discuss this with their health care provider. If they do not have a health care provider, we may be able to refer them to a qualified physician or counselor in their area.

2. If someone requests genetic test results and we have done studies of clinical significance using their sample, we will either have a clinical geneticist call them back or send them a letter telling them that based on the testing that was done on their cheek sample it appears that they do or do not carry a specific genetic marker. We can tell them the name and location of the genetic marker and some basic information in lay terms. We will explain to them the limitations of the testing that was done (as described above) and offer to assist them in locating a clinical geneticist or genetic counselor if needed. Each CBDRP site will have a clinical geneticist available to answer questions. We will explain to the participant that if they are concerned about genetic factors that may be associated with birth defects, regardless of the results, we suggest that they discuss this with their health care provider, and will help them locate a provider if needed.

We have developed a fact sheet that can be sent to anyone requesting information on the genetic testing done as part of the NBDPS (Attachment 26). The fact sheet explains the nature of the testing that will be done on the cheek cells, the limitations of the technology being used; the fact that the results so far have no clinical implications at this time, and that the research is being conducted to generate hypotheses for future study.

3.B.5. Analytic Approach

Using the diagnostic information included in the clinical database, individual defects will be categorized into appropriately homogeneous groups, including the use of isolated and multiple defects. Analysis of risks from a given exposure will be carried out within broad categories, such as all vascular disruption defects and be narrowed to a given defect such as gastroschisis.

Because controls are population-based and randomly selected, all controls can be utilized for any of the subgroup analyses which involve interview information. Additionally, other cases can be compared to the case group of interest in certain analyses, when appropriate.

In some cases, analysis will be hypothesis driven (e.g. the further evaluation of a previously described association between fever and neural tube defects) and in other cases, analysis will be conducted in the search for new risk factors for individual defects (e.g. the evaluation of associations between specific birth defects and newly available prescription drugs). Univariate analysis will be used to look for individual risk factors for specific defects. Multivariate logistic regression will be the major analytic tool used to evaluate confounding and look for best-fit models to explain the observed outcomes.

An important analytic tool will be to look for evidence of gene-environment interaction in the analysis. Genetic information will be obtained using DNA-based polymorphisms; individuals will be classified according to the presence or absence of specific susceptibility alleles, as well as whether they have those alleles in single (heterozygotes) or double dose (homozygotes). Evidence for interaction will be sought in logistic regression modeling using specific interaction terms.

3.B.5.a. Sharing Data

In early discussions among the CBDRP principal investigators, it was decided that the data should be compiled, edited, and coded centrally to ease the difficulties associated with combining data during analysis. It was agreed that CDC was the best place to accomplish this with the assistance of a data manager whose funding comes from all the collaborating CBDRP. The data managers, while located at CDC, assist all CBDRP with the tasks related to combining study data (see Section 6).

The Data Sharing Committee has two representatives from each CBDRP. Each CBDRP has two votes. The committee has established guidelines for access to the compiled interview and biologic data and is responsible for ensuring that the data is shared equitably among the CBDRP. Any researcher interested in using the pooled data for analysis submits a letter of intent and later a more detailed proposal to the committee for review. The committee considers the scientific merit of the proposals and encourages collaboration among the researchers where possible. The committee has also established guidelines for authorship, acknowledgments, and other issues related to the publication of studies using the collective data (Attachment 13A). The

committee will also insure that all proposed research complies with human subjects' requirements. Additional IRB review will be required for: 1) any research involving collaborators outside the CDC/CBDRP group; or 2) any studies which fall outside of the scope of the current protocol.

3.B.5.b. Sample Size and Power

A birth defect is a structural abnormality present at birth. Most, but not all, are included within the range 740.0 to 759.9 of the International Classification of Diseases Ninth Revision (ICD-9). Birth defects, as a group, are relatively common, occurring in 3-5% of all births. Individual birth defects, however, are relatively rare. Conditions within this category include a heterogenous group of outcomes with differing morphogenesis and they cannot be appropriately evaluated as a group. In the past, it has often been difficult to conduct epidemiologic studies because of the relatively small numbers of specific birth defects. Pooling data from the CBDRP maximizes sample size and provides unprecedented power to evaluate potential risk factors for specific birth defects (Attachment 14).

3.B.5.c. Study Participation Rates

Calculation of participation rates is needed to monitor and evaluate the study progress and interpret study outcome. The targeted participation rate is 75% for both cases and controls. So that rates are comparable across sites, it is important for participation rates to be calculated at each CBDRP using the same methods. Eligibility and participation rates are calculated using the definitions included in Attachment 14. Participation rates are calculated for several subcategories of study participants: cases, controls, specific birth defect groups, and by CBDRP.

Participation rates are frequently monitored throughout the study. Specific reasons for not participating are noted (e.g. mother speaks neither English or Spanish, the mother is deceased). Because of the ongoing nature of the NBDPS, calculation of the overall participation rate at any point in time results in artificially low rates. This is because, while the denominator (identified cases and controls) may be timely, the numerator may lag because some eligible participants will still be in the tracing and contact phase of the study and they have yet to be interviewed. For this reason, additional rates will be calculated to enable us to best estimate how the study is

progressing. Since we are aiming to complete maternal interviews within 6 months of the infant's birth, participation rates which are calculated for a period ending 6 months prior should be expected to be more complete. We also complete participation rates for completed birth periods. A birth period includes infants born >=24 months ago since all interviews must be completed by 24 months post EDD. The combination of running monthly participation rates (which will be artificially low) and the rates for completed years give both a long and a short view of the study progress.

4. PARTICIPANTS

Births occurring on or after October 1, 1997 are eligible for inclusion in the NBDPS in all CBDRP except Arkansas, New Jersey, North Carolina and Utah. (The starting date for Arkansas and New Jersey is January 1, 1998 and the starting date for North Carolina and Utah is January 1, 2003). As described in Subpart D of 45 CFR Part 46, this project fulfills the requirements for investigations involving children in that it involves only minimal risk and presents an opportunity to understand and prevent a serious problem affecting the health and welfare of children.

4.A. NBDPS Case Selection

As previously described in Sections 3.A., each of the CBDRP programs conducts population-based birth defects surveillance, monitoring all births occurring to residents in a defined geographic area having at least 35,000 births each year. Following a clinical case review, using specified inclusion and exclusion criteria (Section 3.B.1, Attachment 6) case-infants are selected from among the population of infants ascertained within the individual CBDRP surveillance programs (Attachment 3). Infants who have been diagnosed with at least one of the selected defects (Attachment 5) are eligible for inclusion in the NBDPS. Approximately 300 eligible case-infants will be enrolled in the NBDPS each year at each Center.

4.B. Selection of Control-Infants

Each of the nine CBDRP conducts interviews with approximately 100 mothers of control-infants every year. As described in Section 3.B.2., controls are randomly selected from among all births occurring in the defined geographic area of each CBDRP, using one of two methods.

Researchers in Arkansas, Georgia, Iowa, Massachusetts, North Carolina, New Jersey and Utah select their controls randomly from electronic birth certificate files; California, New York and Texas use hospital data to select controls using a stratified random sample, weighted by the number of births occurring in each of the birth hospitals in that area. Using a random numbers generating program, a list of potential controls is generated, stratified by hospital and month of delivery, with over sampling to allow for about 70% participation rates.

Regardless of which method is used, each birth has the same probability of being selected from within each geographic area (from among the approximately 35,000 to 75,000 yearly births occurring to residents of that specific geographic area). Once a birth has been randomly selected, identifying information is abstracted, including names of the infant, mother and father, address, and date of birth.

4.C. Procedures for Tracing and Contacting Participants

The investigators (and their contractors) have in place an extensive procedure for tracing individuals (Attachment 15) to insure that lost-to-follow-up rates are minimized as much as possible. Once potential participants have been located, one of two initial contact procedures may be used, depending on the CBDRP.

4.C.1. Initial Contact - Physician

For the states with physician contact as a first step, physicians are sent a letter with return receipt requested. The letters inform them of the plan to interview one or more of their patients and ask if there is any reason their patient(s) should not be contacted (example - Attachment 16). If a reply is not received within 21 days, contact is initiated with the mother by an introductory letter (Section 4.C.2., Attachment 18) which explains the purpose of the study and requests their participation.

Occasionally, CBDRP researchers find that not enough specific information from the medical records is available to determine whether or not the birth defect meets the CBDRP case definition and inclusion criteria. When this situation occurs, a letter is sent to the appropriate physician (according to hospital records) asking for assistance (Attachment 17). The letter is followed with a phone call to determine if the physician has additional information on the

specific diagnosis, which they are willing to share with us. Once all available classification information has been obtained, if the infant meets the CBDRP case definition, enrollment in the study is initiated.

4.C.2. Initial Contact - Prospective Participant

Letters of introduction (Attachment 18) are mailed to participants. Initial letters differ, depending on whether the participant is the parent of a case infant or a control infant, and whether there is a known pregnancy termination, fetal death, or infant death. Included with the letter of introduction is a $20 money order, a fact sheet on rights of human subjects (Attachment 18A), a calendar (Attachment 19), food frequency list (Attachment 20), and a study information pamphlet (Attachment 21).

4.C.3. NBDPS Pamphlet

The study pamphlet (Attachment 21) which serves several purposes was designed by members of the CBDRP standards committee and revised in 2003. The pamphlet addresses a number of IRB issues raised by several states and provides general information to participants, physicians and other interested parties. The pamphlet is included with the letter of introduction to potential NBDPS participants, because more information is provided in the pamphlet than might be reasonably included in an introductory letter. The pamphlet includes detailed study information, answers to anticipated questions, and addresses anticipated concerns.

4.D. Telephone Contact - Study Participants

Ten days after the initial letters of introduction have been sent, a follow-up telephone call is made by specially trained interviewers at each CBDRP site to: 1) explain the study; 2) obtain oral informed consent; and 3) set up a convenient time for the conduct of the telephone interview. The NBDPS CATI Interviewer Instructions Manual is included in Attachment 22. A complete description of telephone scripts is included in Attachment 23.

Following the introduction, the interviewer establishes with the participant the expected date of delivery (EDD) and an estimated date of conception (DOC). If the respondent knows the due date, then the CATI instrument automatically calculates the DOC and which pregnancy months correspond with the calendar months. If the mother does not know the due date, the interviewer

will use the previously determined EDD, which was abstracted from medical records or calculated, as necessary following the protocol for determining the estimated date of conception (Attachment 24).

Once the calendar is established, the interview is conducted (Attachment 8), following the question-by-question interviewer instructions (Attachment 22). Interviews are targeted for completion within 6 months of delivery, with a maximum time to interview of 24 months after EDD (or delivery for full term infants). No interviews will be conducted in the first 6 weeks after EDD (or delivery for full term infants). If pregnancies have been electively terminated, interviews will be delayed until six weeks after the EDD, to avoid routinely obtaining interview information earlier for such cases, resulting in potential bias.

For some CBDRP sites, when telephone interviews are not possible (e.g. the participant does not have a phone or has been difficult to locate through routine tracing), in-person interviews may be conducted.

4.D.1. Letter of Thanks

Immediately following completion of the interview, subjects are sent the interview thank you letter (Attachment 27) and a copy of the participant newsletter (Attachment 31).

4.E. E-Mail Contact – Study Participants

At many of the CBDRP, the greatest barrier to participation is locating the mother of the index baby so that we can send her the introductory packet for the study. The process of tracking and tracing mothers relies heavily on identifying a phone number at which the mothers can be reached. There have been many changes since the study's inception affecting this part of the tracking and tracing process primarily that the presence of land lines has decreased and the use of cell phones as the only form of telephone has increased. Cell phones are currently much harder to track because directories are not readily available. Another trend in communication has been the increasing use of e-mail and social networking sites. The Abt tracing staff for the Atlanta site has documented several instances in which a mother's e-mail address was the only form of contact information they could obtain and when a mother could be plausibly identified on a social networking site, but no other contact information is available. These instances were more

common among younger mothers, a group who have traditionally been harder to track and trace in the NBDPS. For this we will use e-mail as a means of contacting mothers in a way equivalent to that currently used for telephone calls. E-mail communication will be treated as analogous to telephone communication when leaving messages on an answering machine. A complete description of the E-mail scripts is included in Attachment 32. These scripts differ depending on whether the participant is the parent of a case infant or a control infant, and whether there is a known pregnancy termination, fetal death, or infant death.

4.F. Request for Cheek Cell Samples

After the interview thank you letter is sent, a buccal (cheek) cell collection kit is sent to the mother to take a sample on her child (if living) and both parents. The collection kits include a letter describing the buccal collection, informed consent forms, a pen, simple instructions, a $20 incentive, materials for completing the specimen collection, a Frequently Asked Questions form, and prepaid U.S. mail packets for specimen return. If after several weeks the completed buccal cell collection kit is not returned, the interviewers will call the mother and send a reminder letter to see if she has any questions about how to complete the kit. If there is still no response, a final letter is sent to the mother encouraging her to complete the kit and stating that if we don't receive the kit 2 weeks hence, we will assume she is not interested in participating.

4.G. Incentives for Cheek Cell Samples

CBDRP sends participants $20 with the buccal (cheek) cell collection kit (Attachment 26).

4.H. Incentive for Completion of the Entire Study

Following the completion of study participation, a letter of thanks is sent to the mother (Attachment 27). Mothers who complete both the interview and the buccal (cheek) cell sample kit are sent an additional $20 money order.

4.H.1. Recollection of buccal cell samples that fail laboratory quality control analyses (Approved 6/3/2004)

The Centralized Laboratory located at CDC performs quality control analyses of NBDPS buccal cell brushes. The majority of samples pass the quality control analyses but occasionally the laboratory is unable to obtain reliable information from some samples. This may be due to a

variety of reasons, such as too little genetic material or contamination of the sample during transit or processing.

On June 3, 2004, we received approval to request additional samples from subjects whose buccal cell samples do not pass these quality control analyses. We approach the subject initially by phone and then by a follow up letter. The following documents were added to the NBDPS protocol.

Buccal Re-Collect Phone Script (Attachment 25D)
Buccal Re-Collect Letter (Attachment 25E)
Buccal Re-Collect Thank You Letter (Attachment 25F)

Currently, NBDPS subjects who receive the original buccal cell packet are compensated with a $20 money order for collecting cheek cells from the mother, father and baby. Additionally, at all Centers, subjects receive another $20 money order once their samples are received at the local Center. We follow this same scheme when issuing the re-collection packets (i.e. a $20 money order is included with the recollection kit and another $20 money order is sent to participants once recollected samples are received at the CBDRP).

4.H.2. Dry Brush Project

Collaborators in Iowa, Georgia and New York completed a pilot study recollecting buccal cytobrushes from families that previously submitted buccal cells using a method that did not allow cytobrushes to dry during transport, referred to as the "wet" brush method. The kitsused for the pilot study contained cytobrushes that are allowed to dry during transport, referred to as the "dry" brush method. Previous studies showed that brushes allowed to dry during transport resulted in better quality and quantity DNA (the NBDPS switched from "wet" brush collection to "dry" brushes in August 2003). In addition, collaborators in Georgia and New York requested samples from those families who participated very early in the study (1997-1998) and were never asked to collect buccal cells.

Targeted subjects had birth years between 1997 and 2002. The case families included had children with spina bifida or longitudinal limb defects. Inclusion criteria for both cases and controls were that the child was still living, they previously contributed a buccal sample (except the GA and NY families from 1997 and 1998), and they had not been asked to resubmit buccal samples previously (In June 2004, the NBDPS received approval to request additional "dry" brush samples from subjects whose buccal cell samples do not pass quality control analyses). There was a 1:1 ratio of cases: controls that was proportional to the total number of eligible cases per birth year.

There were 252 total families asked for a dry brush recollect. The subjects included 64 families from Georgia: 32 controls (including 13 who never contributed buccals) and 32 cases (19 spina bifida including 11 who never contributed buccals; 13 longitudinal limb defects including 2 who never contributed buccals); 98 families from Iowa: 49 controls and 49 cases (36 spina bifida; 13 longitudinal limb defects); 90 families from New York: 57 controls (including 21 who never contributed buccals) and 34 cases (22 spina bifida including 10 who never contributed buccals; 12 longitudinal limb defects including 6 who never contributed buccals).

Pilot study results included a buccal recollect kit return rate in Atlanta of 47% for families who previously sent in a "wet" brush kit and 31% for families who were never asked to collect cheek cells. The return rate in Iowa was 75% for families returning the recollect kits. The return rate in NY was 57% for families who previously sent in a "wet" brush kit and 41% for families who were never asked to collect cheek cells. These participation rates surpassed the minimum 20% rate acceptable for continuation/expansion of this study. In addition, 99% of the recollected samples were categorized as "pass" following quantitative and qualitative analyses on the DNA. This "pass" rate is greater than the 90% pass rate acceptable for continuation/expansion of the study.

Since the pilot study goals for participation and DNA quality control were surpassed, we requested and were granted approval to expand buccal recollection to all remaining collaborating centers that previously had collected "wet" brush samples: AR, CA, MA, and TX.

4.H.3. Biologics Focus Groups

In September 2007, we conducted focus groups to assess the attitudes of both mothers who participated and mothers who did not participate in the collection of biological specimens from themselves, their infants and young children. A total of 38 mothers attended six focus groups comprised of: (1) non-Hispanic Black mothers of case-infants who participated or (2) did not participate in DNA collection, (3) mothers of any race or ethnicity who had case-infants of low birthweight who participated or (4) did not participate in DNA collection, and (5) non-Hispanic Black mothers of control-infants who participated or (6) did not participate in DNA collection. Moderator-led discussions probed maternal attitudes toward providing specimens, factors that influenced decision making, and collection method preferences. The goal was to increase the effectiveness of studies that currently collect biological specimens from infants and their families but with less than optimal response rates, such as the National Birth Defects Prevention Study (NBDPS), and studies that are working to implement the use of biological specimens, such as the Pregnancy Risk Assessment Monitoring System (PRAMS). Results from the qualitative research study have been published. Based on these results, modifications to the NBDPS are being developed that include the addition of an NBDPS website where participants can view a family collecting buccal cells, changes to introductory letters and phone scripts to better explain how participants were selected and contact information was obtained, and the addition of verbiage to cytobrush wrappers to alleviate concerns about potential contamination (e.g., "Sterile" or "Do not use if seal is broken").

4.I. Benefits and Risks to Subjects

Some subjects may be uncomfortable responding to some of the questions (e.g. substance and alcohol use). Respondents (once having agreed to participate) are reminded that they maintain the option of not answering any individual question. It is our experience, however, that mothers of newborns are enthusiastic about participating in studies which elucidate the causes of birth defects. In addition, the interviewer will communicate to participants that we do not know whether there is a link between birth defects and some of the health behaviors and exposures, which are the subject of this research.

The procedure of cheek cell sampling causes little to no discomfort and has a minimal possibility of infection. Risks associated with the genetic research conducted on these samples are minimal because the anticipated genetic research conducted within NBDPS is not, in general, of a sensitive nature. The research performed will relate to highly polymorphic genetic variants. In addition, the risks of disclosure have been minimized through the records handling precautions (Section 6) and the removal of personal identifiers from interviews and biologic specimens.

There are no other immediate risks or benefits to the study subjects. It is our expectation that science and society in general will benefit if we are better able to understand the causes of birth defects; this information may lead to improved intervention and prevention strategies for birth defects.

5. INFORMED CONSENT PROCEDURES

The investigators believe that these surveillance and research activities present no more than minimal risk, and thus are consistent with regulations concerning the protection of human subjects.

5.A. Oral Informed Consent for Interview

For the interview, no written informed consent will be obtained. Oral consent to an interview is obtained prior to conducting the interview. (See page 2, telephone script, Attachment 23.) Any questions a woman may have about the study are answered, and verification of the study may be obtained, if necessary, from the principal investigator at each CBDRP site.

5.B. Written Informed Consent for Genetic Research

Written informed consent is obtained for each participant that agrees to provide cheek cell samples. The standard version (written for Metropolitan Atlanta) is included in Attachment 10. The written informed consent document has been subjected to a number of reading level evaluations; it is at approximately the ninth grade reading level. It includes the following information: the purpose of the study, the procedures, risks and benefits, information on confidentiality, costs, compensation, the participant's right to refuse or withdraw, control, ownership, and commercial value of biologic materials, and phone numbers for questions about the research and about their rights as a human subject.

The written informed consent document informs participants that CBDRP scientists intend to conduct genetic research within the CDC laboratory and/or laboratories within the Centers for Birth Defects Research and Prevention which is directly related to birth defects research. The consent form explicitly states:

> "These samples will be used to study genes that may play a role in why some babies have birth defects. They will only be used to study birth defects and for no other purpose."

If parents cannot read the written consent forms or if they returned a kit without a signed consent form and prefer verbal buccal consent, they are read a verbal consent form.

Occasionally mothers return cheek cells samples without the signed informed consent form. These mothers are then sent another consent form with a request for the appropriate signatures. If, after repeated attempts, the mother does not return the signed consent form, and can no longer be contacted, her family's cheek cells will be included in the study. By returning the cheek cell samples these mothers have implied their consent to participate.

In addition, the informed consent documents (both written and oral) include information regarding the Certificate of Confidentiality and a description of the circumstances under which study information will be shared (Attachments 10, 23, 28).

6. RECORDS MANAGEMENT

6.A. Medical Records Data

The birth defects case records, which include identifying information, are stored permanently in locked file cabinets at each CBDRP site. Information is entered electronically and all data files are password-protected. Access to data files that include personal identifiers (such as names, addresses, telephone numbers, Social Security numbers, and hospital chart numbers) are restricted to staff members who have a need to work with the data and who know the password and data set name. Analytic data are accessible to collaborating scientists who have signed the Confidentiality and Data Use Oath (Attachment 13A, last two pages).

At each site, all data stored on disks are protected by a computer security system that limits access to designated staff that has a legitimate need to access this information because of their official duties involving records processing (updating, correcting, and changing records). These safeguards conform to the privacy act system of records number 09-20-0136, published on page 37718 of the Federal Register on September 25, 1984.

Note: For the Atlanta NBDPS, the MACDP and the original BDRFS are covered by a federal Assurance of Confidentiality (308(d)), which requires all employees, contractors, and students to sign annual confidentiality pledges. All data collected within MACDP, including that used by the Birth Defects Risk Factor Surveillance Project are covered by this Assurance. The coverage extends to all historical data and will extend to all future data. Because of the nature of the collaborative effort, a Certificate of Confidentiality has been obtained for the sites participating in the NBDPS (Section 7; Attachment 28).

6.B. Interview Data

The interviews are administered using a custom SQL database created by the CDC, the CATI database contains all of the screens to guide the interviewer through the entire interview while collecting the data directly into the database. Personal identifiers that are part of the case

interview are stored in a separate database from the interview data. The interview database contains all of the interview's coded responses and is linked to personally identified information only by a nine-digit study identification code. The interview database is configured for replication, a process that will allow the transfer of information to the CDC where the interviews will be collected and maintained centrally. (See Section 3.B.5.a.)

The CDC has dedicated a server to the NBDPS, which receives incoming interview data from the individual CBDRP sites. This server is located in the secure NCBDDD Information Resources Management (IRM) office. The NBDPS Server runs Windows 2000 Advanced Server and has password protected access. The resources of the NBDPS Server are only accessible locally at the server. At the CDC, Chris Cosper, John Sims and Dr. Reefhuis, the CBDRP Project Officer, are currently the only users with local access to the NBDPS Server. Data coordinators at all CBDRP sites send data monthly via the Secure Data Network (SDN).

The process by which individual CBDRP will update information to the NBDPS Server is known as replication, a process that allows multiple copies of the same database to be synchronized so the resulting two databases will be identical. Each CBDRP site keeps only its own data, and CDC maintains a repository of the combined data from all sites.

At monthly intervals, the individual states will use the NBDPS replication tool to transfer their data to the central database via the SDN.

6.C. Clinical Data

The clinical database at each CBDRP contains characteristics of each case and control infant but does not contain personal identifiers. Access to the database is limited to those clinicians and researchers who have direct responsibility for maintaining the database. All employees, contractors and collaborators at any of the CBDRP sites with access have signed pledges of confidentiality. We also track the analytic database to ensure all data is returned at the conclusion of the analysis.

The clinical database will follow the same replication and security process as the interview instrument database (Section 6.B.) It will be linked to the interview database only by the nine-digit study identification number.

6.D. Biologic Specimen Data

Biologic samples obtained as part of this project are stored in a secure manner without identifiers (with the exception of study identification number) in appropriate storage facilities at CBDRP-designated labs and the CDC central biologic specimen repository, as described in Section 3.B.4.c. Banking of Biologic Specimens. A tracking system has been developed at CDC to record the location and history of biologic specimen collection for this study and it includes specific information on the use of individual study specimens. Each CBDRP uses this biological tracking system and again the data is replicated to CDC where the compiled database is maintained.

The NBDPS Server is backed up weekly and taken offsite periodically by the NCBDDD IRM team. Although these data are only identifiable by a nine-digit identification number, it is still maintained with appropriate security measures. The data extracts transferred to CDC do not include names, addresses or other personal identifiers.

6.E. Genotyping Database

The NBDPS is currently looking into establishing a centralized genotyping database. This database will be used as a data management system to store genotyping results centrally, which will allow all collaborators to share data equally. An amendment to this protocol will be done once a potential data management system is determined.

7. CERTIFICATE OF CONFIDENTIALITY

As previously mentioned, a Certificate of Confidentiality has been obtained (Attachment 28) for the CBDRP and the NBDPS. All CBDRP have cooperative agreements with CDC and have individual IRB approval to conduct the NBDPS in their geographic area. All have provided letters of support for the Certificate of Confidentiality (Attachment 29).

Investigators within the CBDRP are not considered outside investigators. It is understood, however, that before outside collaborators (other than CBDRP scientists or their affiliates) are permitted access to personally identifiable data from the NBDPS, special permission must be sought from participants and permission must be granted for any such specified use.

Potential participants are given the following information about the Certificate of Confidentiality:

> All information that we gather in this study will be kept private. This is assured under Section 301(d) of the Public Health Service Act (42 U.S.C. 241(d)). The Certificate of Confidentiality prevents study staff from being forced under a court order or other legal action to identify you or anyone else in this study. Records may be reviewed by officials checking on the quality of the research. This protection lasts forever (even after death) for any persons who were subjects in the research during any time the certificate was in effect. However, you should understand that the investigators are not prevented from reporting information obtained from you to authorities in order to prevent serious harm to yourself or others. Cheek cell samples will be stored without your names, but are linkable. Information about you may be shared with other participating sites and other researchers when and if it has been approved by research review. The shared data will not contain any information that could identify any individual.

8. INTERNAL AND EXTERNAL REVIEWS AND APPROVALS

Documents supporting internal and external reviews and approvals are included in Attachment 30. All CBDRP sites have received approval by their individual IRBs. The original CDC IRB approval for the Atlanta BDRFS was granted on May 14, 1992, with the original authorization to give an Assurance of Confidentiality granted on May 8, 1992. The most recent CDC IRB approval for the Atlanta NBDPS was granted on January 25, 2010 with an expiration date of January 29, 2011. The Certificates of Confidentiality for the NBDPS were awarded to the original CBDRP sites on August 2, 1999 (Attachment 30) and will expire on January 31, 2014.

References

1. Miles AM, Singer PC, Ashley DL, Lynberg MC, Mendola P, Langlois PH, Nuckols JR. Comparison of trihalomethanes in tap water and blood. *Environ Sci Technol*. 2002 Apr 15;36(8):1692-8.

2. Lynberg M, Nuckols JR, Langlois P, Ashley D, Singer P, Mendola P, Wilkes C, Krapfl H, Miles E, Speight V, Lin B, Small L, Miles A, Bonin M, Zeitz P, Tadkod A, Henry J, Forrester MB. Assessing exposure to disinfection by-products in women of reproductive age living in Corpus Christi, Texas, and Cobb county, Georgia: descriptive results and methods. *Environ Health Perspect*. 2001 Jun;109(6):597-604.